PHARMACOGNOSTIC STUDIES ON AFRICAN ERYTHROPHLEUM SPECIES

Anders Cronlund

ERYTHROPHLEUM SUAVEOLENS (Guill. & Perr.) Brenan

University of Uppsala
1975

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In this survey, the following papers will be discussed and will be referred to by the Roman numerals given in the following list. Some unpublished results are also presented.

I A. Cronlund. The botanical and phytochemical differentiation between *Erythrophleum suaveolens* and *E. ivorense*. Submitted to *Planta Medica*

II A. Cronlund and F. Sandberg. New alkaloids from bark of *Erythrophleum ivorense*. *Acta Pharm. Suecica* 8, 351 (1971)

III A. Cronlund. Two new alkaloids from bark of *Erythrophleum ivorense*. *Acta Pharm. Suecica* 10, 507 (1973)

IV A. Cronlund. Conformational isomerism in a new alkaloid from *Erythrophleum suaveolens* syn. *E. guineense*. *Acta Pharm. Suecica* 10, 333 (1973)

V A. Cronlund and James U. Oguakwa. Alkaloids from the bark of *Erythrophleum couminga* Submitted to *Acta Pharm. Suecica*

VI S. Jansson and A. Cronlund. Alkaloids from the bark of *Erythrophleum africanum*. Submitted to *Acta Pharm. Suecica*

VII A. Cronlund. Semisynthetic cassaine analogs. Submitted to *Acta Pharm. Suecica*

VIII A. Cronlund and F. Sandberg. Cardiotonic effect and toxicity of *Erythrophleum* alkaloids. Submitted to *Acta Pharm. Suecica*

IX A. Cronlund. Metabolism of cassaidine in the guinea pig. Submitted to *Acta Pharm. Suecica*

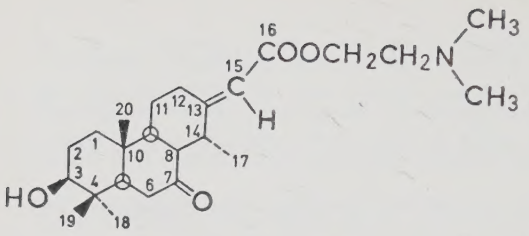
X A. Cronlund. Synthesis and cardiotonic activity of three new cassaine analogs. Submitted to *Acta Pharm. Suecica*

1. Introduction

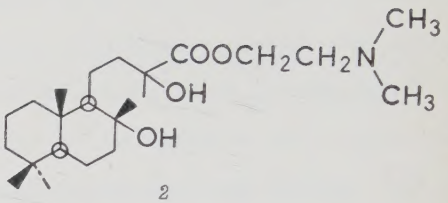
Cardiac glycosides from plants are still the main remedy for treatment of cardiac insufficiency. However, these compounds are highly toxic with an unfavorable therapeutic index and intoxications are common, especially in older people. The research in the field has in later years tackled this problem along two different lines: the development of methods for a more accurate dosage and the search for new compounds with a better therapeutic index.

The first line of research has lead to new assays for cardiac glycosides in blood plasma [1,2,3]. The second line is based on the theory that the therapeutic and toxic effects can be separated, and has resulted in the synthesis of a number of compounds related to the cardiac glycocides. [For a reveiw see 4]. During the search for new structures with a positive inotropic effect, cardiotonic alkaloids have come into focus. A review of cardiotonic alkaloids from *Erythrophleum, Voacanga and Veratrum* was made in 1967 by Thorp and Cobbin [5]. Of these, the *Erythrophleum* alkaloids have been most studied. Since Dalma [6] isolated cassaine (*1*) from the bark of *Erythrophleum suaveolens* in 1939 the structures of about 30 alkaloids have been elucidated (*Table 1*).

Besides the isolation of *Erythrophleum* alkaloids from plant material, new pharmacologically active cassaine analogs have been synthesized. Clarke *et al.* [7] made 4,4,14-tridemethyl-7-deoxo analogs whereas Hauth [8] synthesized a series of 14-demethyl-7--deoxy derivatives. *N,N*-dimethylaminoethyl sclareolate (*2*) prepared from the diterpene sclareol Pope *et al.* [9] is a cardioactive compound related to the *Erythrophleum* alkaloids. An intermediate between the cardiac steroids and the alkaloids is represented by the synthetic substance *3* [10].



1 Cassaine



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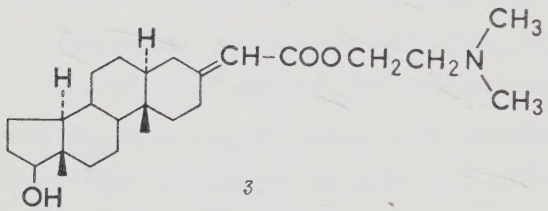

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Along with the isolation and synthesis of new alkaloids, pharmacological screenings using intact animals, isolated hearts and heart strips or the ATP´ase inhibition technique have been performed [5 (review) 11,12,13,14]. All alkaloids with a positive inotropic effect have also, when tested, been shown to be inhibitors of this enzyme (see section 8:4).

From the accumulated data on the structure-activity relationships of these compounds Portius *et al.* [15] in 1964 postulated a digitalis-receptor common to the alkaloids and cardiac steroids. The model was further developed by Repke in 1972 [16] and by Thomas *et al.* [4] in 1974. These authors´ explanation of the structure-activity relationships is that the polycyclic ring skeleton binds the drug close to the receptor, and that the α,β-unsatured ester/lacton is the actual key which triggers the pharmacological response.

Besides the effect on the heart higher doses of *Erythrophleum* alkaloids, like the cardiac glycosides, produce womiting and convulsions . The alkaloids are also reported to be potent local anesthetics [11]and cause a direct non-adrenergic stimulation of vascular smooth muscle [12]. Recently Loder *et al.* [17] reported that norerythrostachaldine and its 3β-acetate are highly cytotoxic.

2. Scope of the present investigation

When this work was started the development of column chromatography on Al_2O_3 had been shown to be a good method for separation of the *Erythrophleum* alkaloids [18]. It was also known that structural variations of the alkaloids at position 3 and at the double bond greatly affected the pharmacological action of the alkaloids. Furthermore there were indications that the cardiotonic and toxic effects of the alkaloids could be separated [19].

The purpose of the investigation summarized here was to extend the knowledge about the relations between structure, cardiotonic activity and toxicity for the *Erythrophleum* alkaloids and to provide some basic data on their metabolism. To obtain a variety of structures for the pharmacological screening, isolation of alkaloids from plant material as well as semisynthetic work should be performed. The barks of *E. suaveolens* and *E. ivorense* are very similar. No good methods for their identification are described and it was therefore necessary to develop a method by which the two barks could easily be botanically differentiated.

The differentiation between barks from *E. suaveolens* and *E. ivorense* is described in paper I. The isolation of new alkaloids from these two barks and from barks of *E. africanum* and *E. couminga* is described in paper II-VI. Semisynthetic derivatives of some isolated alkaloids are presented in paper VII and

X, and the pharmacological screening of isolated and synthesized compounds is presented in papers VIII and X. Paper IX depicts results from metabolic studies of cassaidine.

3. Definitions and nomenclature

The genus *Erythrophleum* was characterized in 1832 by G. Don and under the name of *Fillaea* by Guillmein & Perroret. The most well-known member of this genus is *E. suaveolens* (Guill. & Perr.) Brenan, which in the literature also is described as *E. guineense* [20]. Furthermore the name *E. micranthum* still occurs in the literature altough the correct name *E. ivorense* should be used for this species [21].

The nomenclature of *Erythrophleum* alkaloids is ambigous. In Chemical Abstracts they are indexed as derivatives of phenanthrencarboxylic acid (cassamidine) or derivatives of acetamide (norcassaide).

In the present work the nomenclature of Rowe [22] is followed, when a new compound cannot be named as a derivative of an accepted trivial name .(Since the introduction of the name norcassaidide [23], 3β-acetoxy-7β-hydroxy-*N*-(2-hydroxyethyl)-*N*-methyl--*E*-13(15)-cassen-16-amide (paper IV) can be named as norcassaidide 3β-acetate.) An exception from this rule is the introduction of the amide nomenclature (cassamide, cassaide, erythrophlamide). The reason for introducing this nomenclature is that the strict chemical names are difficult to use by pharmacologists. The trivial names for *Erythrophleum* amides have by Loder *et al.* [24] been named as norderivatives (*i.e.* cassamide is named as norcassamide), and in the later part of the present work this nomenclature is used.

4. Historical remarks on the use of barks from African Erythrophleum species

The use in Africa of aqueous extracts of barks of *Erythrophleum* species as an ordeal poison is wellknown. *E. suaveolens* has been used most frequently especially by tribes in Senegal [25,26,27]. However, the ordeal trial was quite common in Africa and also *E. ivorense* [21], *E.africanum*, *E. lasianthum* [28], and *E. couminga* [27] have been used for the ceremony.

The strong pharmacological actions of plants of this genus combined with the mysticism has led to extensive use of different parts of the trees in local medicine as emetic, purgative, against headache, rheumatism, visceral - and tooth pain and externally against filaria and dermatoses. Plant material or extracts have also been used as parasiticides, fish-,rat- and arrow poisons [21,28,29,30].

5. Botanical identification of the investigated plant material

5:1 Introduction

The genus *Erythrophleum* (Caesalpiniaceae) is found in Asia, Australia and Africa. The characterisation of different species is uncertain [25,31]. However, in Africa the following five species can be distinguished with certainty [32,33,34,35]. *E. suaveolens* (Guill. & Perr.) Brenan, *E. africanum* Harms, *E. ivorense* A. Chevalier, *E. couminga* Baillon and *E. lasianthum* Corbishley[1]. The distribution of these species are shown in *Fig. 1*.

Fig. 1 The geographical distribution of African *Erythrophleum* species.

The differentiation between the first two of these is rather easy [33]and they have also different geographical distribution. *E. couminga* can't be mistaken for any other species since its distribution is limited to Madagascar and the Seychelles where no other *Erythrophleum* species are found. The differentiation between *E. suaveolens* and *E. ivorense* is more uncertain and they both occur in the moist semideciderous forest [25,36,37,38].

5:2 BOTANICAL DIFFERENTIATION BETWEEN *E. SUAVEOLENS* AND *E. IVORENSE* (PAPER I)

Most botanical characters described in the literature were re-investigated on plant material from 9 countries in West Tropical Africa. Of all characters mentioned earlier, locality, the angle of the fruit stake to the ventral suture, the shape of the leaflet and the appearance of secondary nerves were found to be most reliable characters for each specimen.

5:3 PHYTOCHEMICAL DIFFERENTIATION BETWEEN *E. SUAVEOLENS* AND *E. IVORENSE*

It was found (paper I) that barks from *E. suaveolens* and *E. ivorense* could be differentiated according to their different alkaloid content. Ordered with respect to relative amounts *E. suaveolens* contained the following ester alkaloids: Erythrophleguine>>erythrophlamine>cassaine (sometimes cassamidine). Norerythrophlamide was dominant among the amide alkaloids. In *E. ivorense* on the other hand the proportions of the ester alkaloids were

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[1] *E. lasianthum* has not been available in this investigation.

different: cassaine, was dominant followed by cassaidine and erythrophleguine. Several amide alkaloids were present but none was dominating. The phytochemical differentiation facilitates the pharmacognostical field work and constitutes a method for identifying commercial bark samples.

6. Isolation of alkaloids from barks of E. ivorense, E. suaveolens, E. couminga and E. africanum

6:1 INTRODUCTION

Most alkaloids from *Erythrophleum* species have been isolated from stem barks (*Table 1*). However, alkaloids have also been found in the root bark of *E. suaveolens* and *E. ivorense* [paper I], in the leafs of *E. chlorostachys* [3]and in seeds of *E. suaveolens* [25]. Furthermore the roots of *E. africanum*, leafs of *E. suaveolens E. africanum*, *E. lasianthum* [28] and wood of *E. suaveolens* [25] and *E. ivorense* [21] are reported to be toxic. The most toxic part of the trees seems to be the seeds [25,28] whereas the only non-alkaloidal part probably is the pods [28, paper I]. (For non alkaloidal compounds isolated from *Erythrophleum* species see ref. [28].)

6:2 STRUCTURES

6:2:1 The skeleton

All alkaloids hitherto isolated from barks of *Erythrophleum* species are esters or amides of *N*-monomethylaminoethenol or *N*,*N*-dimethyl aminoethanol and different cassenic acid derivatives. [For a rewiev see 31] The amides are probably artefacts [41] formed from the corresponding esters by contact with alkaline solutions during the working up procedure. In *Table 1* all crystalline and some amorphus

Table 1. Alkaloids isolated from barks of *Erythrophleum* species [1]

Mol weight	Name	Molecular formula	Found in	m.p. or α_D	Ref
391	Norcassaide	$C_{23}H_{37}NO_4$	*i,*	205°	II
393	Norcassaidide	$C_{23}H_{39}NO_4$	*ch*	244°	41
405	Cassaine	$C_{24}H_{39}NO_4$	*s,i,co,f*	142°	51
407	Cassaidine	$C_{24}H_{41}NO_4$	*s,i,co,ch*	139°	51
407	Norerythrostachaldine	$C_{23}H_{37}NO_5$	*ch*	HCl 156°	17
407	Norerythrostachaldide	$C_{23}H_{37}NO_5$	*ch*	225-7°	17
419	Norcassamide	$C_{24}H_{37}NO_5$	*s,i*	134°	II
421	Norcassamidine	$C_{24}H_{39}NO_5$	*ch*	—	24,4
421	Norcassamidide	$C_{24}H_{39}NO_5$	*s,ch*	115°	24,4
421	19-Hydroxycassaine	$C_{24}H_{39}NO_5$	*i*	145-7°	III
433	Cassamine	$C_{25}H_{39}NO_5$	*s,i,co*	86-7°	51
433	Dehydronorerythrosuamine	$C_{24}H_{35}NO_6$	*s*	α_D^{20}-41°	52,7
435	Norerythrophlamine	$C_{24}H_{37}NO_6$	*ch*	HCl 212°	43
435	Norerythrophlamide	$C_{24}H_{37}NO_6$	*i,ch,a*	165°	II
435	Cassamidine	$C_{25}H_{41}NO_5$	*s,i,co,ch,a*	HCl 235-6°	18
435	Norerythrosuamide	$C_{24}H_{37}NO_6$	*s*	α_D^{20}-72°	52,7
435	Norcassaidide 3β-acetate	$C_{25}H_{41}NO_5$	*s*	202-4°	IV
437	Norerythrostachamine	$C_{24}H_{39}NO_6$	*ch*	—	41
437	Norerythrostachamide	$C_{24}H_{39}NO_6$	*ch,a*	205°	41
447	Alkaloid A		*a*	105°	IV
449	Erythrophleguine	$C_{25}H_{39}NO_6$	*s,i,co*	77-8°	51
449	Erythrophlamine	$C_{25}H_{39}NO_6$	*s,i,co,a*	149-51°	51
449	Erythrosuamine	$C_{25}H_{39}NO_6$	*s*	HCl 139-41°	18
449	Norerythrostachaldine 3β-acetate	$C_{25}H_{39}NO_6$	*ch*	HCl 231-2°	17
449	Norerythrostachaldide 3β-acetate	$C_{25}H_{39}NO_6$	*ch*	—	17
451	C_{24} Amine	$C_{24}H_{37}NO_7$	*ch*	HCl 191°	43
473	Ivorine	$C_{28}H_{43}NO_5$	*i*	166-7°	48
477	Norerythrophlamine 3β-acetate	$C_{26}H_{39}NO_7$ [3]	*ch*	—	43
479	Norerythrostachamine 3β-acetate	$C_{26}H_{41}NO_7$ [3]	*ch*	—	43
487	3-(3-Methylcrotonoyl)cassaine	$C_{29}H_{45}NO_5$	*i*	169-70°	III
491	Coumingidine	$C_{28}H_{45}NO_6$	*co*	160-1°	56
493	C_{24} Amine 3β-acetate	$C_{26}H_{39}NO_8$ [3]	*ch*	—	43
505	Coumingine	$C_{29}H_{47}NO_6$	*co*	142°	55
507	Coumidine	$C_{29}H_{49}NO_6$	*s,i*	130-2°	18
533	Norcoumingide acetate	$C_{30}H_{47}NO_7$ [3]	*co*	114-5°	V
577	3-Acetoxynorerythrosuamide diacetate	$C_{30}H_{43}NO_{10}$ [3]	*co*	171-2°	V

1) In addition alkaloids with the following molecular weights have been detected in *E. suaveolens*: m/e 375,377,447,477,491 [51] and in *E. couminga*: m/e 419,435,449,493 and 507 [paper V].

2) *i=E. ivorense, s=E. suaveolens, co=E. couminga, ch=E. chlorostachys a=E. africanum*

3) Calculated from structure.

alkaloids isolated from the actual barks are ordered according to their molecular weights.

6:2:2 Hindered rotation around the amide linkage (paper IV)

Studies on the amide bond of norcassaide have been carried out. It was known that the NMR signal from the olefinic proton at C-15 appears as two broad peaks and that the same is true for the $N-CH_3$ signal . In spectra recorded at elevated temperatures, the actual signals appeared as slightly broadened singlets. This shows that the signal pattern at the nonelevated temperature must be assigned to the effect of a barrier to internal rotation in the amide. The same interpretation of the signal pattern of norcassamide has been published [24]. (Similar results have also been shown for *N*-methylformamide and *N*-methylacetamide by Drakenberg *et al.* [42]).

6:3 CHROMATOGRAPHY OF *ERYTHROPHLEUM* ALKALOIDS

TLC on silica gel and aluminium oxide are common methods for separating the *Erythrophleum* alkaloids. Column chromatography has also been carried out on silica gel [23] aluminium oxide (this work) and sephadex [39]. Contercurrent distribution and [43] liquid-liquid column chromatography [44] have also been performed.

Gaschromatography cannot be used for separation of alkaloids with a dimethylaminoethyl arrangement since they decompose at higher temperature. However, GLC has been used for separating methyl or ethyl esters of cassenic acid derivatives [45,46].

6:4 ALKALOIDS FROM *ERYTHROPHLEUM IVORENSE* (PAPER II-III)

6:4:1 Previous work

When this work started the only alkaloid isolated from *E. ivorense* was ivorine [47,48]. Simultaneously with the publication of paper II Schultz *et al.* [49] reported the isolation of

new alkaloids. The correct structure for these compounds were later proposed by Loder *et al.* [50] and shown to be identical with norcassamide (*4*) and norerythrophlamide (*5*).

6:4:2 New alkaloids of the amide type (paper II)

Norcassamide (*4*) norerythrophlamide (*5*) and norcassaide (*6*) were isolated following chromatography on aluminium oxide of a chloroform fraction of an ethyl acetate extract of the bark. Hydrolysis in dilute hydrochloric acid of the three alkaloids afforded the known acids cassamic acid, erythrophlamic acid and cassaic acid respectively (m.p., IR). From all three hydrolysates it was also possible to isolate *N*-methylaminoethanol and identify it as its 3,5-dinitrobensoate.

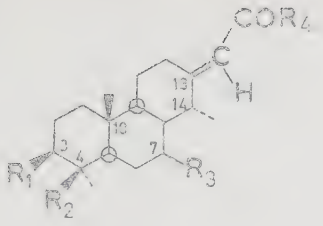
The amide linkage of the alkaloids was established by their relative resistance to hydrolysis in alkaline methanol compared to ester alkaloids and from spectroscopic evidence. The IR spectra showed amide bands rather than ester bands and in the NMR spectra shifts of the $-N-CH_2-$ and $-O-CH_2-$ signals compared to those in spectra of the ester alkaloids were observed.

6:4:3 Ester alkaloids known from other species (paper II)

By column chromatography on aluminium oxide the following known ester alkaloids were isolated from an ether fraction of the extract mentioned under 6:4:2: cassaidine, cassaine, cassamidine, coumidine, erythrophlamine and erythrophleguine. They were identified by their TLC properties and their IR spectra.

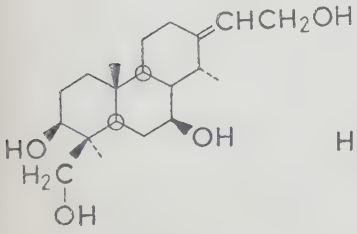
6:4:4 3-(3-Methylcrotonoyl)cassaine (*7*) (paper III)

Rechromatography of Fraction 1, Table 1, in Paper II enabled the isolation of 3-(3-methylcrotonoyl)cassaine (*7*), a *N,N*-dimethylaminoanalog to ivorine. Following acidic hydrolysis the identification of cassaic acid established the skeleton. The



	R_1	R_2	R_3	R_4
Norcassamide	H-	$-COOCH_3$	O=	$-N<^{CH_2CH_2OH}_{CH_3}$
Norerythrophlamide	HO-	$-COOCH_3$	O=	$-N<^{CH_2CH_2OH}_{CH_3}$
Norcassaide	HO-	$-CH_3$	O=	$-N<^{CH_2CH_2OH}_{CH_3}$
3-(3-Methylcrotonoyl)cassaine	$(CH_3)_2C=CHCOO-$	$-CH_3$	O=	$-OCH_2CH_2N(CH_3)_2$
19-Hydroxycassaine	HO-	$-CH_2OH$	O=	$-OCH_2CH_2N(CH_3)_2$
See separate formula				
Norcassaidide 3 -acetate	CH_3COO-	$-CH_3$	O> H	$-N<^{CH_2CH_2OH}_{CH_3}$
Norcoumingide acetate	$(CH_3)_2\overset{OH}{C}CH_2COO-$	$-CH_3$	O=	$-N<^{CH_2CH_2OOCH_3}_{CH_3}$
3-Acetoxynorerythrosuamide diacetate [1]	CH_3COO-	$-COOCH_3$	$-COOCH_3$	$-N<^{CH_2CH_2OOCH_3}_{CH_3}$

[1] This alkaloid has a keto group in position 6.



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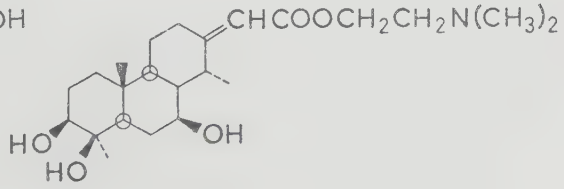

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existence of an ester-bonded *N,N*-dimethylaminoethanol moiety follows from NMR signals of $-N(CH_3)_2$, $-OCH_2-$, $-CH_2-N<$ and from ions in the mass spectrum at M-88 and m/e 58 and 71.

The existence of a 3-methylcrotonoate grouping is supported by its mass spectrum and by signals in the NMR spectrum due to $(CH_3)_2C=$ and a second olefinic proton. In the IR spectrum two bands due to absorption from double bonds occur. Through the partial synthesis of 3-(3-methylcrotonoyl)cassaine (paper VII) the structure was definitely established.

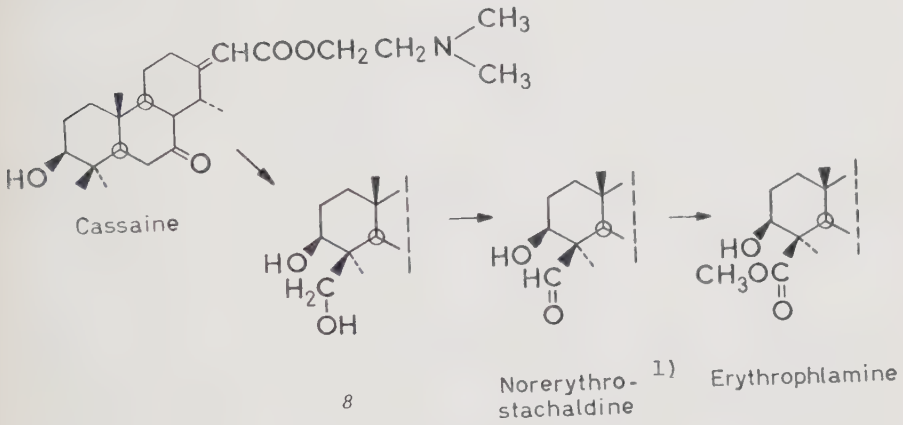
6:4:5 19-Hydroxycassaine (*8*) (paper III)

This alkaloid was isolated from a purified methylene dichloride extract of the bark from another batch than that under 6:4:2. Precipitated alkaloid bisulphates, prepared in the usual manner to isolate cassaine, was converted to bases and subjected to column chromatography (Al_2O_3). Rechromatography of a fraction rich in the new alkaloid allowed its isolation.

The NMR and mass spectra showed that the alkaloid (*8*) was an *Erythrophleum* alkaloid of the *N,N*-dimethylaminoethanol ester type. But NMR also indicated that there was only one methyl group at C-4 which was not accompanied by any C-4 carbomethoxy group. However, hydrolysis of the alkaloid afforded an acid, whose reduced methylester had the same structure (*9*) as reduced methyl erythrophlamate. The IR spectrum of the new alkaloid showed a second carbonyl band besides that of the α,β-unsatured ester, and ORD measurements, with cassaic acid as a reference, established its position at C-7. The IR spectrum of the alkaloid and the NMR spectrum of a prepared acetyl derivative suggested the presence of two hydroxyl groups in the molecule. Their positions followed from the reduced tetrol.

19-Hydroxycassaine represents an oxidation step between

cassaine and erythrophlamine. After the isolation of norerythrostachaldine [17,43] a hypothetical route, as shown in *Scheme 1*, for the biosynthesis of the carbomethoxy group in the *Erythrophleum* alkaloids can be suggested.



Scheme 1

1) Norerythrostachaldine carries a hydroxyl group at position 7.

6:5 ALKALOIDS FROM *ERYTHROPHLEUM SUAVEOLENS* (PAPER IV)

6:5:1 Previous work

Alkaloids isolated from *E. suaveolens* up to 1968 have been reviewed by Manske [51]. Since that time Thorell *et al.* [18] and Friedrich-Fiechtl *et al.* [52] have isolated new alkaloids. From acid hydrolysates Clarke [44] has isolated 8-dehydrocassaic acid and Blessington *et al.* [53] have confirmed the structure of erythrosuamic acid.

6:5:2 Norcassaidide 3β-acetate* (*10*)

Rechromatography on an aluminium oxide column of fractions MI-MIV in paper [18] enabled the isolation of norcassaidide 3β-acetate (*10*). It is the first isolated alkaloid in a series of 3β-acetates [43]. The skeleton of this compound was established by isolation of cassaidic acid from an acidic hydrolysate of the alkaloid. The presence of the acetyl and *N*-monomethylaminoethanol moieties was shown by NMR and mass spectra of the compound. The isolation of cassaic acid from a hydrolysate of the oxidized alkaloid proved the position of the acetyl group at C-3.

6:6 ALKALOIDS FROM *ERYTHROPHLEUM COUMINGA* (PAPER V)

6:6:1 Previous work

Before this work started coumingine had been isolated by Dalma, coumingidine by Schlittler [51] and cassaine and cassaidine by Ruzicka *et al.* [54].

6:6:2 Alkaloids known from other species

From a purified dichloromethane extract of *E. couminga* cassamidine, cassamine, erythrophlamine and erythrophleguine (m.p., IR) were isolated following column chromatography (Al_2O_3). Interpretation of chromatographic properties and mass spectra of two fractions suggested that norcassamide and nor-erythrophlamide were also present in the extract.

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* In paper IV this compound was named as 3β-acetoxy-7β-hydroxy-*N*-(2-hydroxyethyl)-*N*-methyl-*E*-13(15)-cassen-16-amide. See section 3.

6:6:3 Norcoumingide acetate (*11*)

Column chromatography of the extract under 6:6:2 gave a fraction (fraction 5, Table 2, paper V) with one main alkaloid which could not be induced to crystallize. After acetylation of this fraction it was possible to isolate the main acetate, norcoumingide acetate (*11*), by preparative TLC. The basic structure of this alkaloid follows from the isolation of cassaic acid from an acidic hydrolysate of the acetate. The presence of a β-hydroxyisovaleriate grouping at position 3 and an amide bonded 2-(*N*-methylamino)ethylacetate moiety followed from interpretation of mass- and NMR spectra of the acetate. The resistance to acetylation of the β-hydroxyisovaleriate grouping is remarkable. This phenomenon has been observed earlier [55].

Norcoumingide which must be the alkaloid that did not chrystallize, is probably an artefact of the corresponding ester alkaloid (see section 6:2). The previously known ester alkaloid coumingidine has the same molecular weight as norcoumingide.

6:6:4 3-Acetoxynorerythrosuamide diacetate (*1%*, tentative)

This compound was isolated by the methods described under 6:6:3. The NMR spectrum resembled those of other *Erythrophleum* amide alkaloids with a carbomethoxy group at C-4. The mass spectrum suggested the loss of acetyl groups and a 2-(*N*-methylamino)ethylacetate grouping. The amide character of the compound was supported by its IR spectrum, and the appearence of three acetyl groups in NMR indicated the existence of three hydroxyl groups in the molecule.

The 7-hydroxy-6-one arrangement is proposed on spectroscopic evidence. The higher shift in the NMR spectrum of the C(14)-CH_3 signal as compared to other *Erythrophleum* alkaloids is also observed in the 7-hydroxy -6- one substituted alkaloid erythrosuamine. The signals from C(7α)-H and C(3α)-H corresponds also in shape and shift to those reported earlier for 3β and 7β acetates [18, 17]. Furthermore UV measurements and the mass spectrum of the corresponding acid showed that a 6-hydroxy-7-one system was not present.

A new alkaloid, tentatively assigned as 3β-hydroxy norerythrosuamine has recently been isolated by Loder *et al.* (personal communication).

6:6:5 Alkaloid C

Column chromatography and preparative TLC of the chloroform fraction designed 2C in paper V (Table 3) gave small amounts of a new chrystalline alkaloid, which easily decomposes. The mass and NMR spectra suggest the presence of an ester bonded *N,N*-dimethylaminoethanol moiety in the molecule. In the NMR spectrum, which resembles those of other *Erythrophleum* alkaloids, there are only three three-proton signals, assigned to C(14)-CH_3, C(10)-CH_3 and C(4)-CH_3 in the cassen skeleton. The chemical shift of the latter signal (δ 1.27) can be explained by a 3,4-dihydroxy arrangement in the molecule. Such compounds are very easily dehydrated [67] and should show a weak molecular ion in the mass spectrum and an intense M-18 peak. However, the peak (m/e 391) with the highest mass number in the mass spectrum of alkaloid C is not accompanied by any peak 18 mass units below. A possible interpretation is that the observed ion has arisen from a 3,4-dihydroxynorcassen-16-oate

derivative through dehydration. The molecular formula of alkaloid C (m/e 391) as suggested by high resolution mass spectroscopy is $C_{23}H_{37}NO_4$. The NMR spectrum of the isolated alkaloid does not fit this formula but if the actual ion is regarded as a M-18 ion 4-hydroxy-19-norcassaidine (*13*) could be proposed as representing alkaloid C. In the NMR spectrum of a 4ß-hydroxy-19-norditerpene derivative like (*13*) one would expect the three-proton singlet for C(10)-CH_3 to appear at a higher shift than the corresponding signal of the *gem*-dimethyl analog (i.e. cassaidine). Such a shift was not found in the spectrum of alkaloid C. It is possible to assigne alkaloid C as a 4-hydroxy-18-norcassen-16-oate derivative, but all isolated *Erythrophleum* alkaloids have had the 18-methyl group intact.

Since alkaloids with an aldehyde group at position 19 recently have been isolated [43] and since such aldehydes are easily oxidized to 4-hydroxy-norditerpenes [67], alkaloid C may be an artefact formed during the working up procedure.

6:6:6 Other alkaloids

In the extract (6:6:2) alkaloids with the following molecular weights were detected 413, 419, 449, 493 and 507 together with three new alkaloids designed A_1, A_2 and B.

6:7 ALKALOIDS FROM *ERYTHROPHLEUM AFRICANUM* (PAPER VI)

6:7:1 Alkaloids known from other species

Individual alkaloids have previously not been isolated from *E.africanum* Harms. In the present work cassamidine, erythrophlamine, norerythrophlamide and norerythrostachamide were isolated. The structures of the first three followed from comparisons with

authentic samples, whereas that of the fourth followed from the reduction of norerythrophlamide. The separation of the alkaloids followed essentially the procedure described in paper II.

6:7:2 Alkaloid A

This alkaloid was isolated by column chromatography (Al_2O_3) together with an alkaloid with chromatographic properties like cassamine and could be separated from the latter by TLC on Al_2O_3. Mass and NMR spectra suggested that the compound belonged to the ester series of *Erythrophleum* alkaloids with a *N,N*-dimethylamino-ethanol side-chain at C-J6 and a carbomethoxy group at position 4. Furthermore the molecular ion M^+447 allows two carbonyl groups to be attatched to the skeleton. An amorphus alkaloid with such a skeleton has been isolated from *E. suaveolens* [52].

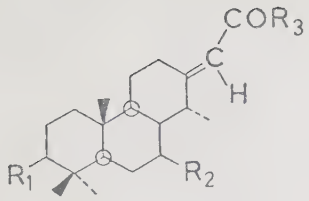
7. Semisynthetic cassaine analogs (papers VII and X)

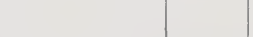
7:1 Introduction

An introduction to previous work has been given in section 1. The structures of the compounds reported below were confirmed by identification of known acids from acidic hydrolysates and by interpretation of mass, IR and NMR spectra. For compounds which couldn't easily be transformed to known alkaloids elemental analysis were carried out.

7:2 3-(3-Chlorpropionyl)cassaine, 3-(O-Chlorbenzoyl)cassaine, 3-(3-Methylcrotonoyl) cassaine

These compounds were synthesized from cassaine (*1*) and the apropriate acid chloride. 3-(3-Methylcrotonoyl)cassaine (*7*) has also been isolated from *E. ivorense* (see 6:4:4).



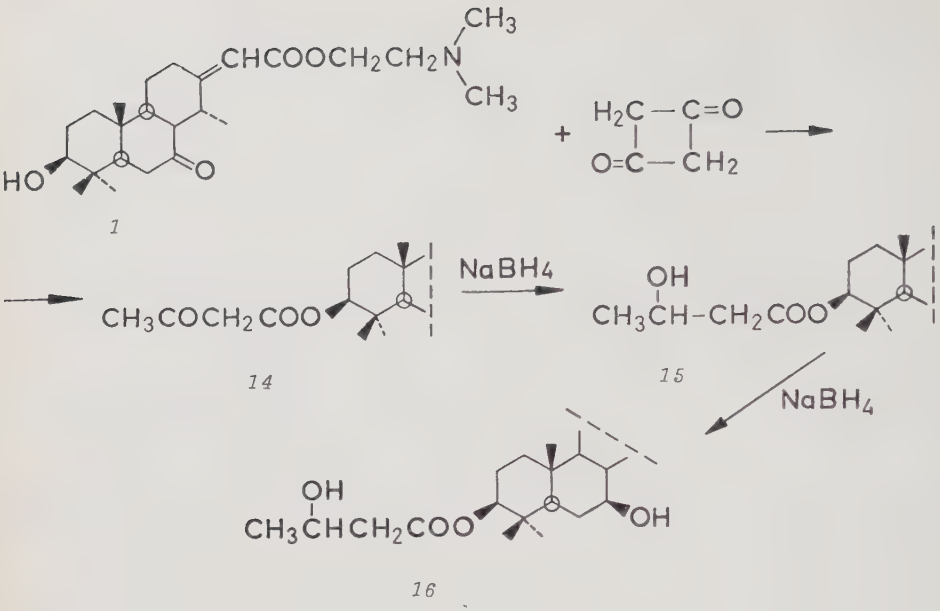
		R_1	R_2	R_3
14	Acetoacetylcassaine	CH_3COCH_2COO-	O=	$-OCH_2CH_2N(CH_3)_2$
15	3-(3-Hydroxybuturyl)cassaine	$CH_3CHOHCH_2COO-$	O=	"
16	3-(3-Hydroxybuturyl)cassaidine	"		"
17	Methylcassaidine			
18	γ-*N*,*N*-Dimethylaminopropyl 3β,7β-dihydroxy-*E*-13(15)--cassen-16-oate			
19	16-O-γ-*N*,*N*-Dimethylaminopropyl 19-O-methyl 7-oxo-13(15)--cassen-16,19-dioate		O=	"
20	β-*N*,*N*-Dimethylaminoethyl 3,7-dioxo-*E*-13(15)-cassen-16-amide	O=	O=	

7:3 ACETOACETYLCASSAINE (*14*), 3-(3-HYDROXYBUTURYL)CASSAINE (*15*) AND 3-(3-HYDROXYBUTURYL)CASSAIDINE(*16*)

Acetoacetylcassaine was synthesized from cassaine and diketene. 3-(3-hydroxybuturyl)cassaine was then prepared by selective reductic of (*14*). As shown in *Scheme 2*,3-(3-hydroxybuturyl)cassaidine was prepared by complete reduction with $NaBH_4$ of (*14*). Partial hydrolysis of 3-(3-hydroxybuturyl)cassaine afforded a new acid, assigned as 3-(3-hydroxybuturyl)cassaic acid.

7:4 METHYLCASSAIDINE (*17*), γ-*N*,*N*-DIMETHYLAMINOPROPYL 3β, 7β-DIHYDROXY-*E*-13(15)-CASSEN-16-OATE (*18*), 16-O-γ-*N*,*N*-DIMETHYLAMINOPROPYL 19-O-METHYL-7-OXO-*E*-13(15)-cassen-16, 19-DIOATE (*19*) AND β-*N*,*N*-DIMETHYLAMINOETHYL 3,7-DIOXO-*E*-13(15)-CASSEN-16-AMIDE (*20*).

The ester alkaloids (*17*, *18*, *19*) and the amide alkaloid (*20*)



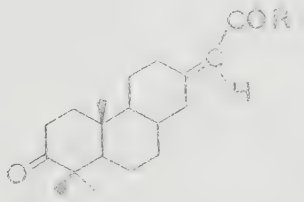
were synthesized by a method described by Hauth [57]. An amino-alcohol or an amine was allowed to react with the acid chloride of 3-dehydrocassaic acid, except in the preparation of (*19*) where the acid chloride of cassamic acid was used. *N,N*-dimethylaminopropanol was used for the preparation of (*17*), (*18*) and (*19*). In the preparation of (*20*) *N,N*-dimethylaminoethylamine was used. Cassaic acid is a *trans* double bond isomer. That this configuration was not affected in the actual reaction was shown by comparition of NMR data of (*18*) with those of *cis*-methyl cassaidate [51].

7:5 7-(*N,N*-DIMETHYLAMINO)-2-METHYL-HEPT-2-EN-4-ON (*21*) AND rac-γ-*N,N*-DIMETHYLAMINOPROPYL-14-DEMETHYL 3-OXO-*E*-13(15)-CASSEN-16-OATE (*22a*).

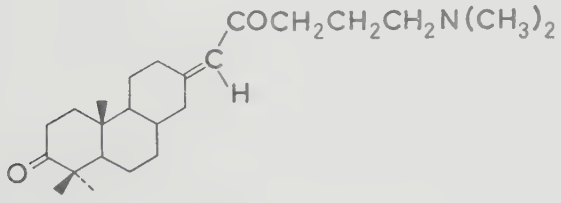
7:5:1 Introduction

Since,as will be shown (section 9), the short duration

of the cardiotonic activity of the *Erythrophleum* alkaloids is probably due to hydrolysis of the ester linkage, the synthesis of the ketone (*23*) seemed interesting. To develop optimal reaction conditions for the preparation of (*23*) the synthesis of the model ketone (*21*) from 3-methylcrotonic acid was performed.



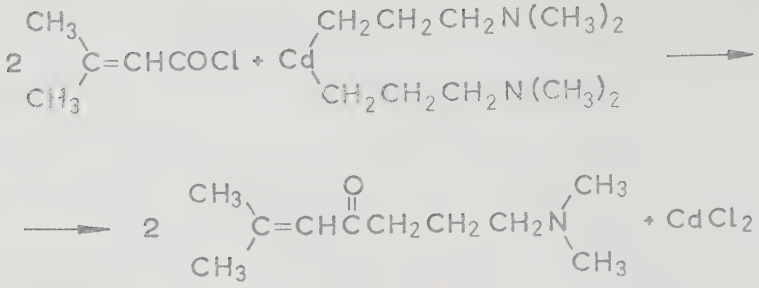
21	See *scheme 3*	
22a	rac-γ-*N*,*N*-Dimethylaminopropyl--14-demethyl 3-oxo-*E*-13(15)--cassen-16-oate	$-OCH_2CH_2CH_2N(CH_3)_2$
22b	rac-14-Demethyl-3oxo-*E*-13(15)--cassen-16-oic-acid	-OH



23

7:5:2 Route of synthesis

Since aliphatic α,β-unsatured ketones had previously been synthesised from acid chlorides and organic cadmium reagents [58] this route shown in *Scheme 3*, was tested for (*21*) with good results. The cadmium reagent was prepared via a known Grignard reagent [59] and the acid chloride from $SOCl_2$ and the actual acid.



Scheme 3 *21*

Although (*21*) was obtained in a high yield, only traces of a compound with a molecular weight corresponding to (*23*) was formed when rac-14-demethyl-3-oxo-*E*-13(15)-cassen-16-oic-acid (*22b*) was used as starting material. Instead rac-γ-*N*,*N*-dimethylaminopropyl-14-demethyl 3-oxo-*E*-13(15)-cassen-16-oate (*22a*), was formed as a major product.

Since Heilman [60] found that impurities in acid halides prepared with $SOCl_2$ inhibits the reaction with organic cadmium reagents, and since it was not possible to purify the acid chloride of (*22b*), impurities in the acid chloride may have disturbed the synthesis of the ketone (*23*).

8. Cardiotonic effect, toxicity and ATP'ase inhibition of Erythrophleum alkaloids (paper VIII)

8:1 Introduction

In this section some biological data are discussed for in this work isolated (section 6) and synthesized (section 7) alkaloids.

It was not possible to test some of the isolated alkaloids due to shortage of material. Likewise some amides were not tested since amides already tested hadn't shown any cardiotonic effect.

8:2 THE RELATION OF CARDIOTONIC EFFECT TO CHANGES IN THE STRUCTURE

8:2:1 The diterpene skeleton

The effect on cardiotonic potency of hydroxylation of the diterpene skeleton varies. It was found, contrary to earlier theories [5] , that even alkaloids without an oxygen function at position 3 also exhibited cardiotonic action. (It should be observed that also 3-desoxydigitoxigenin has a cardiotonic effect [61]). However, 3-hydroxylation increased the potency markedly (compare [7]) while 19-hydroxylation, as in 19-hydroxycassaine, decreased it. 6-Hydroxylation (erythrophleguine) did not effect the potency in a tested compound.

Esterification of the hydroxyl group at C-3 can lead to both increased and decreased potency depending on the nature of the acyl group (see also ref. [5] and [8]). The most potent members of a series of 3-esterified compounds were coumidine, coumingine and 3-(3-hydroxybuturyl)cassaidine (*16*).

Of the tested alkaloids, three pairs differ only by carrying a hydroxyl or a carbonyl group at position 7. The hydroxyl substituent was found to give the most potent compounds. The importance of the presence of an oxygen function at position 7 has been studied by Hauth [8]. Cassaine was about five times as potent as racemic 7-deoxycassaine in the used test system.

Earlier biological screening of cassaine analogs [7] suggested that substitution of the ester linkage for an amide bond caused the

loss of cardiotonic activity. This was supported by testing four new amides: norcassamide (*4*), norerythrophlamide (*5*), norcassaide (*6*) and β-*N*,*N*-dimethylaminoethyl 3,7-dioxo-*E*-13(15)-cassen-16-amide (*20*).

8:2:2 The side-chain

Elongation of the aminoethyl side-chain in cassadine and cassamine to aminopropyl led to decreased activity, although to a lesser extent in the latter case. Since Clarke [7] found an aminobutyl cassaine analog to be equipotent to a semipotent aminoethyl analog a hypothesis that the length of the side-chain is critical only for more potent alkaloids may be formed. It should be observed that rac-γ-*N*,*N*-dimethylaminopropyl-14-demethyl-3-oxo-*E*-13(15)-cassen-16-oate (*22a*) didn't show any activity up to 24 μM/l (paper X).

Metabolic studies (section 9) showed that the alkaloids are rapidly hydrolysed *in vivo*. An attempt to prolong the duration is represented by the synthesis of methylcassaidine (*17*). Its cardiotonic effect was just slightly lowered compared to cassaidine.

Whether a secondary or tertiary alkaloid is the most potent compound has not been thoroughly investigated. The only report is by Schlittler [56], who found the secondary alkaloid coumingidine to be more potent than coumingine. Quaternarisation of the nitrogen causes loss of activity [14].

8:3 TOXICITY AND CARDIOTONIC ACTION

Earlier comparitions of the toxicity of *Erythrophleum* alkaloids have been carried out on coumingine, cassaine and cassaidine by Chen *et al.* [62], and by Santi [11]. In cats the former found

coumingine to be most potent, followed by cassaidine and cassaine, whereas Santi found cassaidine to be most toxic to rabbits followed by coumingine and cassaine.

The present study was performed on guinea pigs and the results were in accordance with the findings by Chen *et al.* Furthermore it was found that both 3-hydroxylation of 3-deoxoalkaloids and acylation of 3-hydroxylated alkaloids with β-hydroxyisovaleric acid could lead to more favourable relations between toxicity and cardiotonic action. Special attention should be drawn to erythrophlamine, which is both more potent and less toxic than cassaine. Among the cardiac glycosides, it has also been possible to differentiate therapeutic and toxic properties by chemical modification of gitoxin to 16-epigitoxin [63].

8:4 ATP´ase INHIBITION AND CARDIOTONIC ACTIVITY

The ability of the *Erythrophleum* alkaloids to inhibit ATP´ase have been investigated by several authors [15, 64, 65]. The question of whether there is a parallelism between inhibition of Na^+, K^+ ATP´ase and positive inotropic effect has been discussed. Professor Repke has tested the effects of some of the alkaloids of Table 2 paper VIII on the enzyme. In *Table 2* (unpublished results) a comparison af ATP´ase inhibition and cardiotonic action for a number of alkaloids is shown. Except for cassamidine there is a good correlation of these two effects.

9. METABOLISM OF CASSAIDINE IN THE GUINEA PIG (PAPER IX)

9:1 EXCRETION OF METABOLITES IN THE URINE

Kahn has reported that cassaine is rapidly inactivated in the dog [12].

Table 2. Correlation between the cardiotonic effect and inhibition of Na^+, K^+. ATP´ase for some *Erythrophleum* alkaloids.

	Cardiotonic effect[1]		Inhibition of Na^+, K^+ ATP´ase[2]	
Most Potent	g-Strophantine	(0,17)		
	Coumidine	(0,38)	Coumidine	(0,016)
			Cassamidine	(0,28)
	Cassaidine	(1,4)	Cassaidine	(0,40)
	Cassaine	(3,1)	Cassaine	(0,50)
	Cassamidine	(3,7)		
	Cassamine	(8,0)	Cassamine	(0,55)
	Erythrophleguine	(8,1)	g-Strophantine	(0,60)
Least potent			Erythrophleguine	(0,88)

[1] µmol/l to produce 70% increase in contractility on papillary muscle *in vitro* (paper VIII) are given within brackets.

[2] µmol/l to produce 50% inhibition are given within brackets.

In the present study similar findings were found for the duration of cassaidine in the guinea pig. With labeled cassaidine it was shown that 85% of the administered radioactivity was eleminated within 15 hours and that cassaidic acid was a main metabolite in the urine. Zelck [66] studied the metabolism of cassaine *in vitro* and found that it is hydrolysed by liver enzymes to give cassaic acid.

9:2 BLOOD LEVELS OF CASSAIDINE AND ITS METABOLITES

The radioactivity resulting from intraveneous administration of cassaidine-7α-T to guinea pigs doesn´t appear in the urine as rapidly as it disappears from the blood.

This suggests that the drug is metabolised or stored outside the blood, and can support Zelck's [66] theory that the alkaloids are metabolised in the liver.

By preparative TLC of extracted blood samples it was possible to determine change with time of levels of unhydrolysed cassaidine and cassaidic acid in blood. The level of cassaidine decreases extremely rapidly the first minutes, and the main metabolite formed is cassaidic acid (*Fig.2*). The inactivation of cassaidine between 2.5 and 40 minutes was determined to about 0.007 µmol/kg body weight and minute. Kahn [12] estimated the inactivation of cassaine, by contineous infusion studies in the dog, to be 0.008-0.012 µmol/kg body weight and minute.

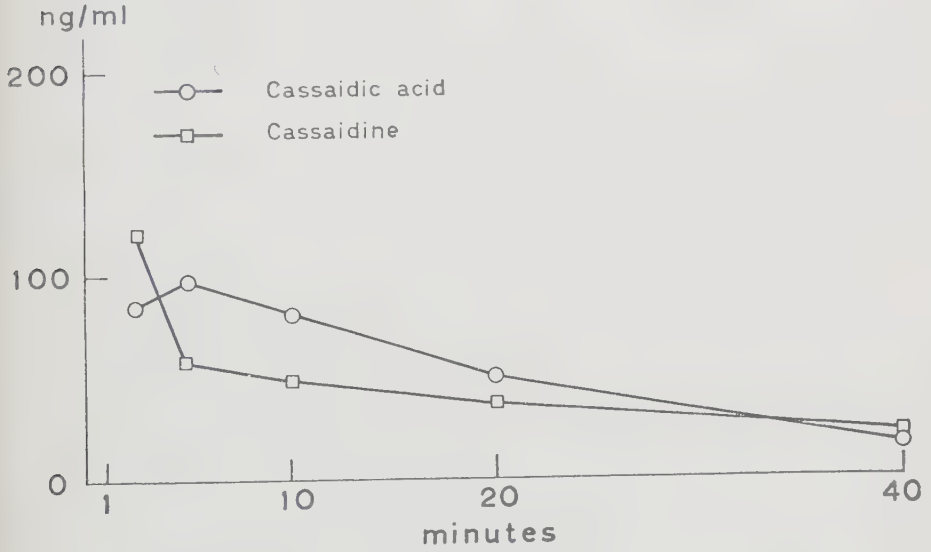


Fig. 2 Levels of cassaidine and cassaidic acid in blood of guinea pig after *i.v.* injection of cassaidine -7α-T HCL (0,70 mg/kg).

However, since Zelck [66] found microsomal homogenates from the guinea pig to hydrolyse cassaine more efficiently than those from the dog, the two species may have different mechanisms for inactivation of *Erythrophleum* alkaloids.

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